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Type I Interferon Is a Powerful Inhibitor of *in Vivo* HIV-1 Infection and Preserves Human CD4$^+$ T Cells from Virus-Induced Depletion in SCID Mice Transplanted with Human Cells

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Although several studies are available on the *in vitro* inhibitory activities of type I interferon (IFN) on HIV-1 replication, the role of these cytokines in the pathogenesis of AIDS is still matter of conjecture. Both beneficial and adverse effects have been envisaged and considered as a possible rationale for the development of either IFN or anti-IFN therapies in HIV-1-infected patients. In the present study, we have evaluated the efficacy of human type I IFN on HIV-1 infection and virus-induced depletion of human CD4 T cells in two models established in SCID mice. In SCID mice transplanted with human U937 cells (U937-SCID mouse model), continuous treatment with type I consensus IFN (CIFN) resulted in a total suppression of HIV-1 infection. This inhibitory effect was superior to that obtained after AZT treatments. Results from an ensemble of experiments in SCID mice transplanted with either control or genetically modified human U937 cells transduced with a Tat-inducible IFN-α gene (LTR-IFN-A2 U937) indicated that low levels of IFN-α, produced locally as a result of virus infection, were extremely effective in inhibiting acute HIV infection and virus replication. Of interest, LTR-IFN-A2 U937 cells conferred a strong anti-HIV-1 protection to coinjected bystander U937 cells. Notably, experiments with SCID mice reconstituted with human PBL (hu-PBL-SCID mouse model) showed that treatment with CIFN inhibited HIV-1 replication more effectively than AZT treatment. Remarkably, treatment with CIFN resulted in a clear-cut protection from the virus-induced depletion of human CD4 T cells, which was also associated with the generation of an antibody response toward HIV-1 antigens in 50% of the virus-injected xenografts. These results suggest that type I IFN efficiently preserves human CD4$^+$ cells from virus-induced damage in hu-PBL-SCID mice, not only by inducing an antiviral state in target cells but also by stimulating anti-HIV-1 human immune responses *in vivo.* © 1999 Academic Press

INTRODUCTION

Several groups have reported that type I interferon (IFN) inhibits HIV-1 replication in human cells cultivated *in vitro* (Bednarik *et al.,* 1989; Dolei *et al.,* 1986; Gendelman *et al.,* 1990; Hartshorn *et al.,* 1987; Kornbluth *et al.,* 1989; Poli *et al.,* 1989; Shirazi and Pitha, 1992). These studies have pointed out the existence of multiple mechanisms by which IFN can affect the HIV infectious cycle, depending on the target cells and the timing of IFN addition and virus infection. In the light of the anti-HIV activities observed in certain *in vitro* cell systems, some clinical trials have been performed with type I IFN (especially α), alone or in combination with anti-HIV drugs, in infected patients (Fernandez-Cruz *et al.,* 1995; Frissen *et al.,* 1994; Kovacs *et al.,* 1996; Lane *et al.,* 1988, 1990; Oka *et al.,* 1989; Rivero *et al.,* 1994, 1997; Skillman *et al.,* 1996). Although a beneficial effect was observed in some of these trials, especially when patients were treated extensively at early stages of HIV disease (Lane *et al.,* 1990; Rivero *et al.,* 1997; Skillman *et al.,* 1996), there is no general agreement on the possible advantage of a clinical use of IFN. Thus, the role of this cytokine in the pathogenesis of HIV-1 infection is still matter of conjecture. In particular, the fact that IFN (commonly termed acid labile IFN-α) is generally present in the serum of HIV-1-infected patients at late stages of the disease (Lau and Williams, 1990) has led to the suggestion that this cytokine can play a role in the progression to AIDS. Moreover, some authors have suggested that type I IFN (especially IFN-α) is an important cofactor in HIV-1 disease, which is directly involved in the suppression of CD4 T cell functions (Fall *et al.,* 1995; Gringeri *et al.,* 1994, 1995; Lachgar and Bizzini, 1994). This assumption is mostly based on the observation that IFN-α can inhibit, under certain *in vitro* conditions, the proliferation of activated T cells and that HIV-1 proteins (i.e., tat) can induce the production of this cytokine (Zagury *et al.,* 1998). Recently, clinical applications aimed at neutralizing the endogenously produced IFN-α in HIV-1-infected patients have begun (Gringeri *et al.,* 1994, 1995). Notably, however, recent studies have shown that the transduction of the IFN-β gene into human PBL results not only in inhibition of HIV replication but also in an enhancement of the development of a potentially protective T cell

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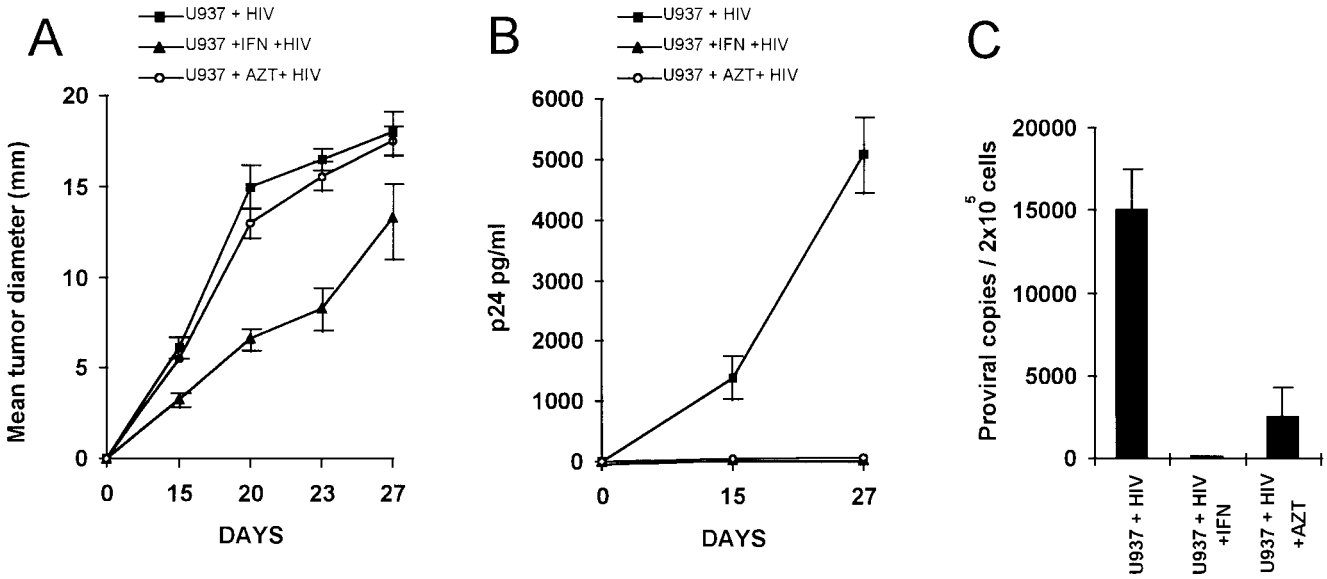

FIG. 1. Inhibition of HIV infection in SCID mice transplanted with U937 cells after repeated treatments with type I IFN (CIFN) or AZT. (A and B) Effect of either CIFN or AZT on tumor growth (A) and p24 antigenemia (B) in SCID mice transplanted sc with U937 cells infected *in vivo* with HIV-1. Mice were injected at the same time with 2×10^7 U937 cells together with HIV-1 (IIIB 10^3 $TCID_{50}$). (■) Untreated SCID mice; (▲) SCID mice treated with CIFN (10^5 U/0.2 ml/mouse, sc; daily, from day −1 to day +25); (○) SCID mice treated with AZT (9 mg/ml/mouse, ip; daily, from day −1 to day +25). (C) HIV-1 DNA PCR analysis of U937 tumor tissues dissected at day 30 after the simultaneous injection of U937 cells and HIV-1 (proviral copy number/2×10^5 tumor U937 cells). DNA was extracted by standard procedure and amplified for gag-specific sequences (Rizza *et al.,* 1996). Sensitivity of the assay was 10 proviral DNA copies, as tested by amplifying DNA, prepared from 8E5-T-cells (Folks *et al.,* 1986), which was serially diluted into SCID mouse cell DNA, as described elsewhere (Lapenta *et al.,* 1997; Rizza *et al.,* 1996). There were six mice in each group.

immunity (Vieillard *et al.,* 1997). Since both IFN-α and IFN-β bind to the same receptor and share many biological properties, these results might suggest that, in general, type I IFN can play beneficial, rather than adverse, effects on T cell functions in the course of HIV-1 infection. Thus, contrasting views may coexist at this stage of our knowledge on the role of type I IFN in the pathogenesis of HIV-1 infection. In this regard, studies on animal models would be helpful for the evaluation of the *in vivo* role of type I IFN in HIV-1 infection and virus-induced depletion of human CD4 T cells.

In the present study, we have evaluated the efficacy of human type I IFN in affecting HIV-1 infection in different models of SCID mice transplanted with human cells. Results from an ensemble of experiments carried out in SCID mice transplanted with parental or genetically modified human cell lines show that type I IFN effectively prevents acute HIV infection and inhibits HIV replication, when it is produced locally at the infection site. Furthermore, data of experiments with chimeric models of SCID mice reconstituted with human PBL (Mosier *et al.,* 1991) have shown not only an impressive type I consensus IFN (CIFN)-induced inhibition of HIV infection (superior to that obtained with AZT treatments) but also a clear-cut protection from the virus-induced depletion of human CD4 T cells. Altogether, these results indicate that *in vivo* type I IFN does not contribute to HIV-induced depletion of CD4 T cells, but it effectively counteracts the deleterious effects of virus infection, by directly acting on the human target cells and, possibly, by enhancing human anti-HIV-1 immune responses in the reconstituted SCID mice.

RESULTS

Repeated treatments of SCID mice with exogenous CIFN completely inhibit HIV-1 infection of transplanted human U937 cell tumors

Coinjection of SCID mice with human U937 cells and HIV-1 results in a progressive infection of the transplanted cells as revealed by the appearance of increasing levels of virus antigen (p24) in the serum and HIV-1 expression at the tumor site (Lapenta *et al.,* 1997). Figure 1B shows the kinetics of development of p24 antigenemia in SCID mice injected with U937 cells and HIV. Daily treatments for 25 days with 10^5 units of type I CIFN starting from the day before virus infection resulted in a complete suppression of the development of p24 antigenemia (Fig. 1B). Likewise, AZT treatment also resulted in inhibition of p24 antigenemia (Fig. 1B). CIFN treatment was associated with a significant delay in tumor growth compared to control treated and untreated mice (Fig. 1A). As illustrated in Fig. 1C, the PCR analysis of freshly dissected U937 cells from IFN-treated mice did not reveal the presence of HIV-1 infection at the tumor site, while only a reduction in the HIV proviral copy number was observed in mice treated with AZT.

TABLE 1

Effect of the Simultaneous Injection of SCID Mice with Either LTR-IFN-A2 U937 or Control U937 Cells Together with HIV on the Development of p24 Antigenemia and the Extent of HIV Infection at the Tumor Site[a]

Cells injected	Mouse No.	Serum p24 (pg/ml) Day 15	Serum p24 (pg/ml) Day 24	*In vitro* p24[b] (pg/ml)	*In vitro* IFN[c] (U/ml)	HIV$^+$ cells[d] (%)
U937	1	≥16,000	≥80,000	≥20,000	<4	36.6
	2	≥16,000	≥80,000	≥20,000	<4	63.3
	3	≥16,000	≥80,000	≥20,000	<4	65.5
LTR-IFN-A2	1	112	1,280	20,000	256	23.0
U937	2	Below detection	200	325	256	1.6
	3	Below detection	140	370	256	2.3

[a] Five-week-old female SCID were injected sc with 10^7 cells together with HIV IIIB (m.o.i. 10^5 $TCID_{50}$) (day 0). On day 15, serum aliquots from tagged mice in each group were tested for p24 levels. Mice were sacrificed on day 24 and the extent of virus infection at this time point was evaluated by testing the serum p24 levels and the level of HIV-1 infection at the tumor site. All the sacrificed mice showed comparable tumors on day 24 (mean diameter 20 ± 2 mm).

[b] p24 released in the culture medium from the freshly dissociated tumor cells after 24 h of culture.

[c] IFN released in the culture medium from freshly dissociated tumor cells after 24 h of culture.

[d] Immunostaining for HIV p55/18 matrix protein of the freshly dissociated tumor cells on day 24.

U937 cells transduced with the Tat-inducible IFN-A2 gene are strongly protected from HIV-1 infection after transplantation into SCID mice: Role of virus-induced IFN-A

In the experiments described above, we had to start injecting 10^5 units of CIFN 1 day before virus inoculation in order to achieve total protection from HIV-1 infection of the U937 cell transplanted SCID mice. Injection of lower doses of exogenous CIFN (10^4 U/mouse) resulted in partial protection from HIV-1 infection (data not shown). It was of interest to evaluate the possible effects of low levels of endogenous IFN produced locally as a result of virus infection. To address this issue, we used U937 cells containing integrated HIV-1 LTR-IFNA2 hybrid plasmids (Su *et al.,* 1995). In the present study, we used a cloned population of cells (ULTR5-14). These cells produced low constitutive levels of IFNA *in vitro,* whose production was significantly increased 2–3 weeks after HIV-1 infection (Su *et al.,* 1995). HIV-1 (pLN4-3) replication was almost completely inhibited for 25 days postinfection (Su *et al.,* 1995). We therefore compared the extent of HIV-1 infection in LTR-IFNA2 U937 cells and control transduced cells when transplanted into SCID mice. Table 1 shows the results of a representative experiment in which SCID mice were injected simultaneously with the genetically modified U937 cells and HIV-1. On days 15 and 24 after infection, all the mice transplanted with control U937 showed high levels of serum p24, while no detectable or low levels of antigenemia were observed in mice injected with LTR-IFNA2 U937 cells and HIV-1. The evaluation of the extent of HIV-1 infection in freshly recovered U937 cells on day 24 after infection revealed a clear-cut inhibition of HIV-1 replication in LTR-IFNA2 U937 with respect to control U937 cells, as shown by the capability of the cells to produce HIV *in vitro* and to express viral antigens (Table 1). Figure 2 shows a typical example of the marked difference in the number of HIV-1-positive cells observed by performing immunostaining techniques with the two types of U937 cell populations. Notably, even though no IFN activity was found in the sera of HIV-1-infected mice injected with LTR-IFNA2 U937 cells, low levels of human IFN were consistently detected in the 24-h supernatants of recovered tumor cells cultivated *in vitro* (Table 1). No IFN activity was found in the supernatants of control U937 cells (recovered from the injected mice), which produced large amounts of HIV-1 (Table 1). These results suggested that even low levels of IFN produced locally after HIV-1 infection can effectively suppress multiple cycles of HIV-1 infection and replication.

We then undertook a set of experiments aimed at determining whether LTR-IFNA2 U937 cells could protect control parental U937 cells from HIV-1 infection *in vivo.* As shown in Fig. 3B, LTR-IFNA2 U937 cells totally inhibited the development of HIV antigenemia when coinjected with control U937 cells, clearly indicating that the IFN produced by LTR-IFNA2 U937 cells as a response to HIV infection can effectively suppress virus replication not only in these cells but also in the bystander parental cells. In this experiment (in which a very low m.o.i. of HIV-1 was used), tumors developed in mice transplanted with either LTR-IFNA2 U937 cells alone or with a mixture of LTR-IFNA2 and parental U937 cells. However, none of the LTR-IFNA2 or the mixed LTR-IFNA2/parental U937 tumors showed the presence of HIV-1, as tested by PCR analysis of DNA extracted from the corresponding tumors (Fig. 3C) and by RT-PCR to detect HIV-1 RNA (data not shown).

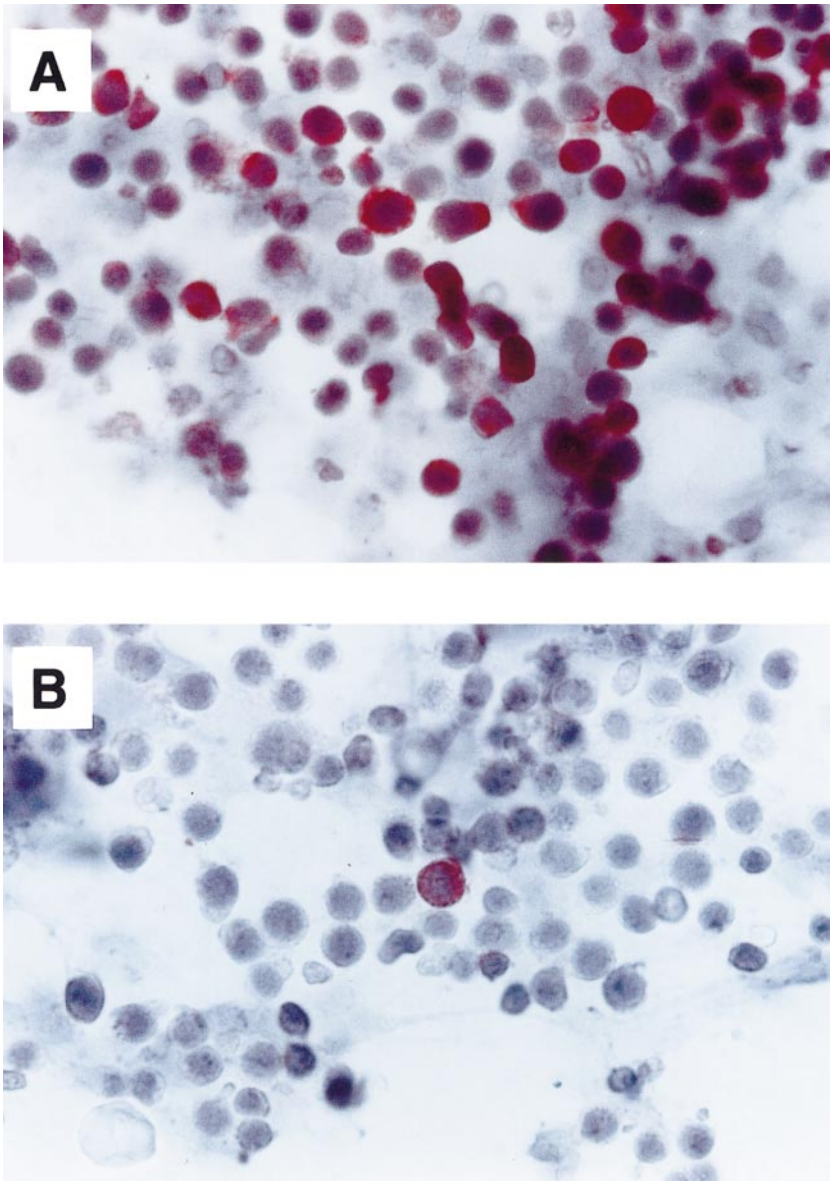

FIG. 2. Detection of HIV-1 p55/18-expressing cells in freshly dissected tumors from SCID mice transplanted with LTR-IFNA2 U937 or control transduced cells and infected with HIV-1. (A) Tumors from mice transplanted with control transduced U937 cells. (B) Tumors from mice transplanted with LTR-IFNA2 U937 cells. Magnification, 650×.

Figure 4 shows the results of two representative experiments in which we injected HIV-1 in SCID mice bearing established U937 cell tumors. SCID mice bearing U937 cell tumors of comparable size (15 days after transplantation) were injected sc with HIV-1. The animals were sacrificed 2 weeks later to determine the serum p24 levels and the presence of virus in the freshly dissociated tumor cells cultivated *in vitro* for 24 h. As shown in Fig. 4, a marked inhibition of HIV-1 antigenemia was observed in mice transplanted with LTR-IFNA2 U937 cells when compared to the animals inoculated with control U937 cells. This suppression in p24 levels was consistent with the inhibition of HIV production observed in the LTR-IFNA2 U937 tumor cells cultured for 24 h *in vitro*.

Protection from HIV-1 infection and CD4 T cell depletion in SCID mice reconstituted with human PBL

The use of the hu-PBL-SCID mouse model allows one to analyze *in vivo* not only HIV-1 infection but also the HIV-mediated immune dysfunction and particularly virus-induced CD4 T cell depletion (Mosier *et al.,* 1991, 1993; Rizza *et al.,* 1996). We have therefore evaluated the effects of CIFN treatment on HIV-1 infection in the hu-PBL-SCID model and compared it to the response obtained after administration of a reference anti-HIV drug (AZT). The hu-PBL-SCID mice were treated daily with either type I CIFN or AZT (starting 13 days after reconstitution with human cells) and infected with HIV-1 on day 14.

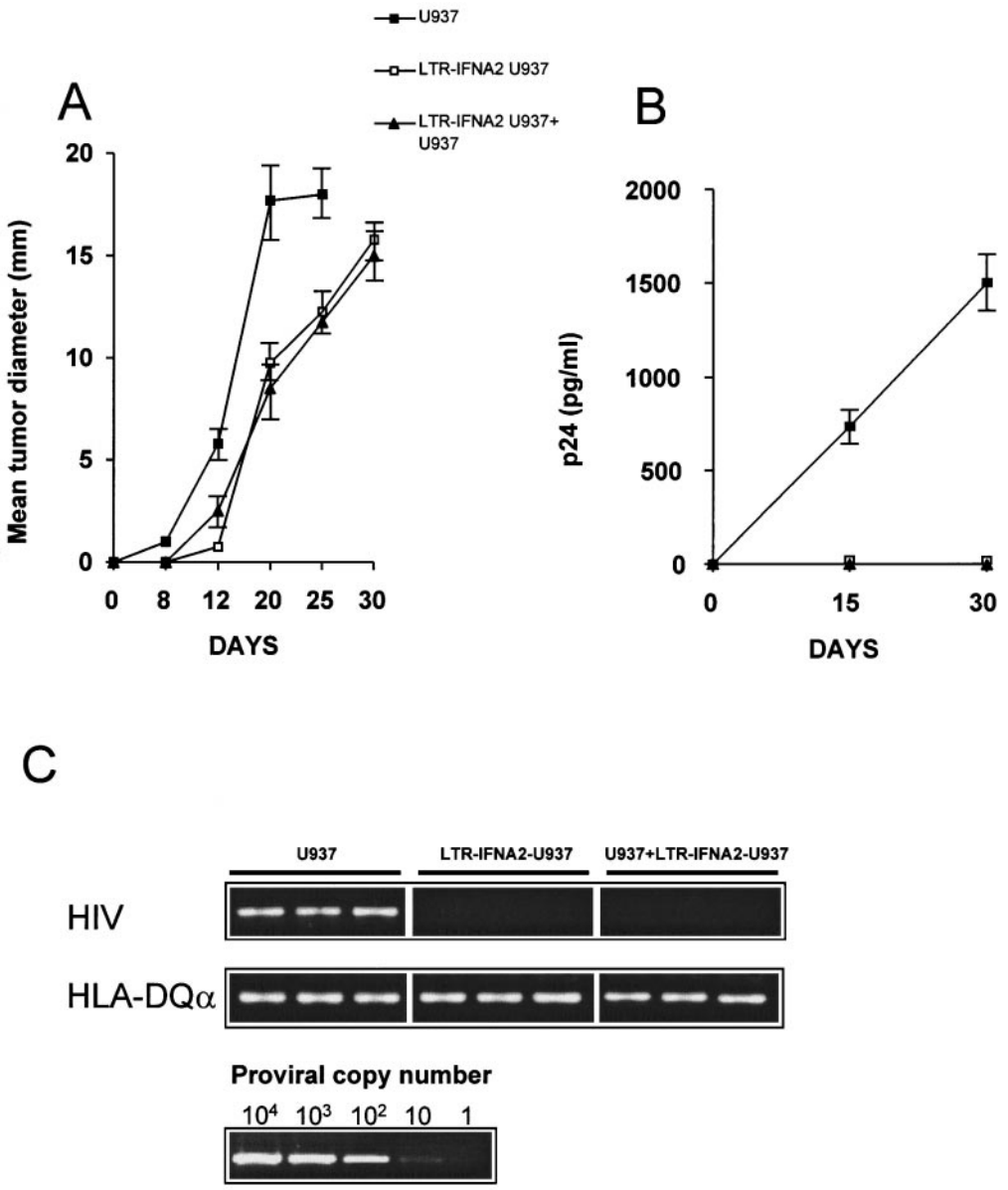

FIG. 3. Protection from HIV infection in SCID mice transplanted with LTR-IFNα2A U937 cells together with control U937 cells. (A and B) Tumor growth (A) and p24 antigenemia (B) in SCID mice transplanted sc with: (■) U937 cells (2×10^7 cells), (□) LTR-IFNA2 U937 cells (2×10^7 cells), (▲) 10^7 LTR-IFNA2 U937 cells together with the same number of control U937 cells. Cells were mixed with HIV-1 (IIIB, 10^3 $TCID_{50}$), before injection into SCID mice. There were six mice in each group. (C) HIV-1 DNA PCR analysis of U937 tumors from SCID mice inoculated with U937 cells as described above. DNA was extracted (at day 30 after the injection with U937 cells and HIV-1) by standard procedures and amplified for gag-specific sequences (Rizza *et al.,* 1996). The PCR results for three representative mice per group are shown. Sensitivity of the assay was tested by amplifying DNA, prepared from 8E5-T-cells (Folks *et al.,* 1986), which was serially diluted into SCID mouse cell DNA, as described elsewhere (Lapenta *et al.,* 1997; Rizza *et al.,* 1996).

Animals were sacrificed 2 weeks later and the extent of HIV-1 infection and CD4 depletion was determined. As shown in Figs. 5A and 5B, treatment with CIFN resulted in a total suppression of HIV replication measured by the HIV-1 plasma RNA copy number and by the levels of HIV p24 in the peritoneal washings. AZT also suppressed HIV-1 replication, but the inhibition was lower than in CIFN-treated animals (Figs. 5A and 5B). Notably, the lymph nodes and spleen from all the hu-PBL-SCID mice infected with HIV-1 and treated with CIFN showed a high level of reconstitution with human cells (as evaluated by PCR for HLA-DQα) and no presence of viral infection (as revealed by PCR analysis for HIV DNA) (data not shown). Of interest, anti-HIV-1 antibodies were detected in the serum of virus-infected hu-PBL-SCID mice treated with CIFN (Fig. 5C).

Figure 6 shows the percentages of human $CD4^+$ and $CD8^+$ T cells found in the different groups of

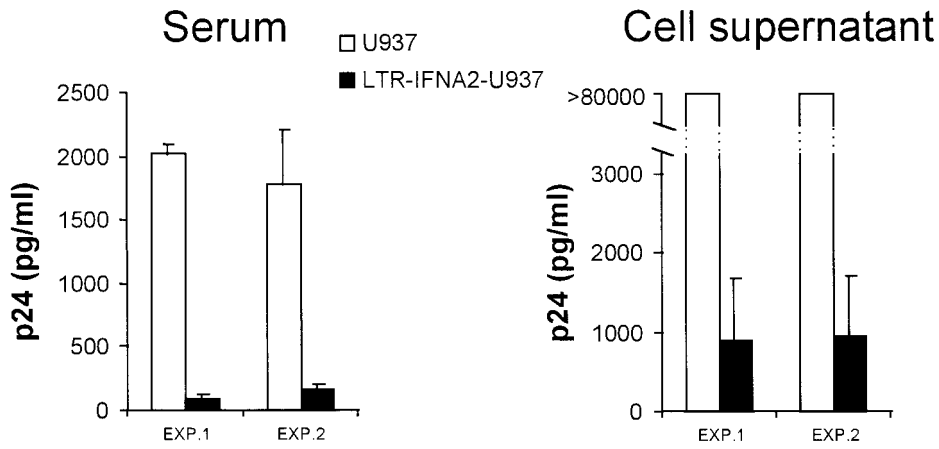

FIG. 4. Effects of HIV-1 infection in SCID mice bearing either control or LTR-IFNA2 U937 cell tumors on the development of p24 antigenemia. Six-week-old female SCID mice were injected sc with 2×10^7 U937 cells as indicated. Mice were treated with anti-granulocyte mAb as described under Materials and Methods. On day 15, mice bearing comparable sc tumors (mean tumor diameter 8 ± 2 mm) were injected with HIV-1 IIIB (10^6 $TCID_{50}$) intratumorally. Two weeks later, the mice were sacrificed and the extent of HIV-1 infection was evaluated by testing: (i) p24 antigenemia in the collected serum; (ii) the levels of *in vitro* p24 production by the U937 cells dissociated from the sc tumors and maintained in culture for 24 h. There were five mice in each group. (□) Mice injected with control U937 cells. (■) Mice injected with LTR-IFN-A2 U937 cells.

hu-PBL-SCID mice in two representative experiments. The infection of control hu-PBL-SCID mice with HIV resulted in a clear-cut depletion of human CD4 T cells. In contrast, HIV-infected mice treated with CIFN showed high levels of human CD4 T cells, which were comparable or even higher than in control uninfected animals (Fig. 6, experiments 1 and 2, respectively). AZT also protected hu-PBL-SCID mice from the HIV-1-induced depletion of human CD4 T cells, but this protective effect was always lower than that observed in animals treated with CIFN. Remarkably, 50% of the HIV-1-injected hu-PBL-SCID mice treated with IFN showed the presence of anti-HIV-1 human antibodies (IgG), while no specific antibodies were detected in the other groups of mice (data not shown).

As shown in Fig. 7, protection from virus-induced CD4 T cell depletion was also observed when CIFN treatment was discontinued on day 7 after HIV infec-

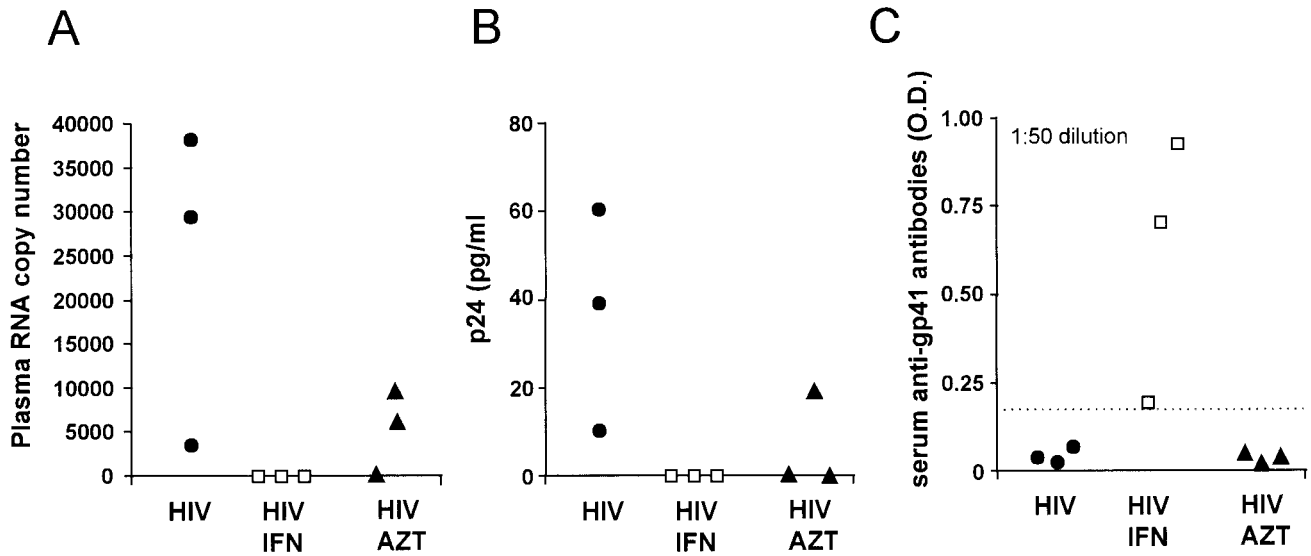

FIG. 5. Suppression of HIV-1 infection by CIFN in hu-PBL-SCID mice: comparison with the effects induced by AZT. Five-week-old female SCID mice were reconstituted with human PBL as described under Materials and Methods. Starting on day 13, mice were daily treated with either CIFN (10^5 U/mouse, sc) or AZT (9 mg/mouse, ip). Hu-PBL-SCID mice were infected on day 14 with HIV-1 SF-162 (10^3 $TCID_{50}$, ip). Mice were sacrificed 2 weeks after HIV-1 infection. HIV-1 RNA copy number in the plasma p24 levels in the peritoneal washings and anti-HIV-1 antibodies in the sera were determined as described under Materials and Methods. Values from three individual mice from each group are shown. Similar results were obtained in two other experiments.

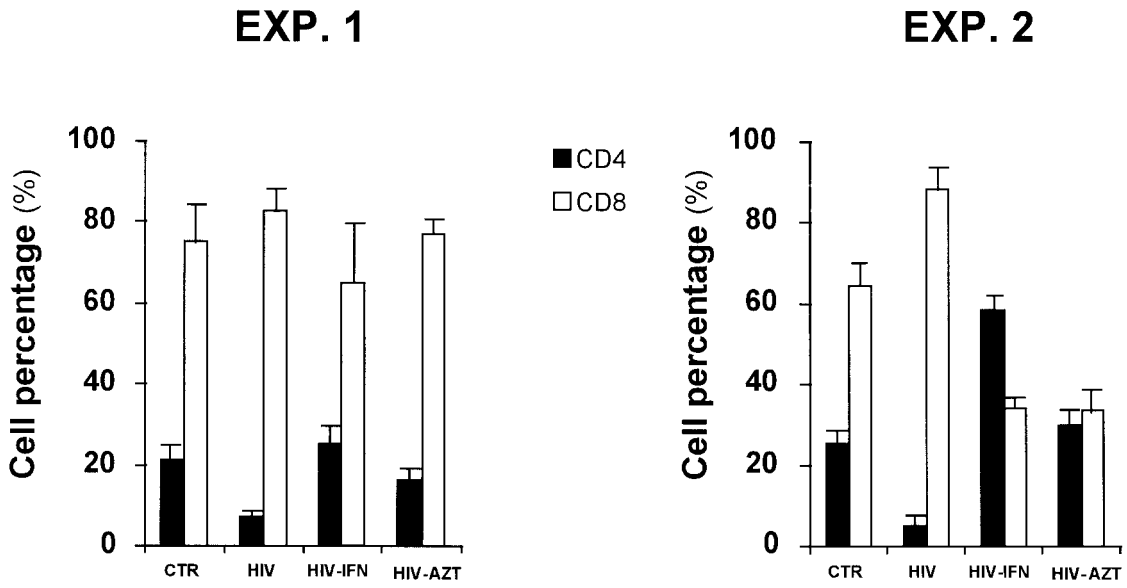

FIG. 6. Persistence of high levels of human CD4 T cells in hu-PBL-SCID mice infected with HIV-1 and treated with type I CIFN: comparison with the effects of AZT. Hu-PBL-SCID mice were treated daily with either CIFN or AZT and infected with HIV-1 (SF162) as described in the legend to Fig. 5. Two weeks after infection, the mice were sacrificed and the percentages of human $CD4^+$ and $CD8^+$ T cells recovered in the peritoneal washings were determined as described under Materials and Methods. There were four mice in each group. Values of two representative experiments of four are shown.

tion. Notably, HIV-infected hu-PBL-SCID mice treated with CIFN exhibited much higher levels of IgM than control mice and a direct correlation was generally observed between the percentages of human CD4 T cells and levels of human IgM in the sera of the injected mice. Of interest, in this experiment the sera of two of four mice infected with HIV-1 and treated with CIFN showed the presence of specific antibodies to HIV-1, while none of control mice developed anti-HIV-1 antibodies (data not shown).

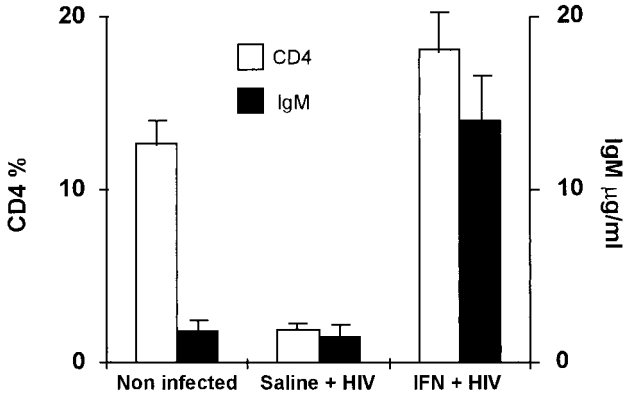

FIG. 7. Persistence of high levels of human CD4 T cells and human IgM in HIV-1-infected hu-PBL-SCID mice in which the IFN treatment was discontinued. Hu-PBL-SCID mice were treated daily sc with 0.2 ml of either CIFN (10^5 U/mouse) or saline, from day 8 to day 16. Mice were injected with HIV-1 (SF162, 10^3 $TCID_{50}$, ip) on day 9. The animals were sacrificed on day 35. The percentages of human $CD4^+$T cells and the levels of human IgM were determined in the peritoneal washings of individual mice. There were four mice in each group.

DISCUSSION

The results reported in this article demonstrate that type I IFN can act as a powerful suppressor of HIV-1 replication in human cells transplanted into SCID mice and can efficiently preserve human CD4 T cells from the deleterious effects induced by virus infection. In both models used in this study (U937-SCID and hu-PBL-SCID mouse models), the protective effects against HIV-1 infection obtained after IFN treatment were more marked than those observed after AZT administration. We have previously shown that cells permanently transfected with a Tat-inducible IFNA2 expression plasmid are not permissive to HIV-1 replication (Bednarik *et al.,* 1989; Su *et al.,* 1995). The use of genetically modified LTR-IFNA2 U937 cells in SCID mice allowed us to evaluate *in vivo* the efficacy of low levels of endogenous IFN-α, which was produced locally as a result of virus infection. Recently, Sanhadji and colleagues have also shown that the transfer of Tat-inducible IFN genes protects human cell lines against HIV-1 challenge *in vitro* as well as *in vivo* after transplantation into SCID mice (Sanhadji *et al.,* 1997). In our study, the finding that a high level of protection from both HIV-1 antigenemia and virus infection was observed in mice transplanted with LTR-IFNA2 U937 cells compared to animals injected with control cells emphasizes the concept that even very low levels of endogenously produced IFN are extremely effective in inhibition of HIV-1 replication. In particular, the fact that LTR-IFNA2 U937 cells were capable of conferring a potent protection against HIV infection when coinjected

with parental virus permissive U937 cells strongly suggests that the virus-induced IFN-α can represent a powerful amplification system for inducing anti-HIV protection in bystander cells, probably by a combination of autocrine and paracrine mechanisms at the infection site. These results appear to be consistent with results by others showing that even very low levels of IFN-β produced by genetically modified human lymphocytes can efficiently prevent HIV-1 infection and replication (Vieillard *et al.,* 1995).

While the results obtained in the U937-SCID models point out the impressive efficacy of type I IFN in preventing HIV-1 infection of human cells under *in vivo* conditions, the data in the hu-PBL-SCID mouse model presented in this article may also shed some light on the controversial issue of the possible involvement of IFN-α in the HIV-1-induced depletion of human CD4 T cells (Zagury *et al.,* 1998). In fact, our results are in contrast with the hypothesis that CIFN can induce CD4 T cell depletion and demonstrate that this cytokine can play a role in maintaining high levels of human CD4 T cells in the virus-infected SCID mice reconstituted with human PBL. In some experiments (Fig. 6, experiment 2), we even observed an increase in the total number of human CD4 T cells in CIFN-treated, HIV-1-infected hu-PBL-SCID mice when compared to the numbers detected in control uninfected mice reconstituted with human PBL. In all instances, the protective effects of CIFN on HIV-1 infection and human CD4 T cell depletion were superior to those found in mice treated with optimal doses of AZT. Notably, the finding that repeated high doses of CIFN in uninfected hu-PBL-SCID mice did not result in any suppression of the levels of human CD4 T cells indicates that, even in the absence of HIV-1 infection, CIFN does not suppress the levels of CD4 T cells. Of interest, a strong protection from HIV-1-induced depletion of CD4 T cells was also observed several days after discontinuation of CIFN treatment (Fig. 7). Notably, hu-PBL-SCID mice treated with IFN showed a marked enhancement in the serum human IgM levels, compared to the control groups, which paralleled high levels of human CD4 T cells (Fig. 7). Interestingly, CIFN-treated HIV-infected hu-PBL-SCID mice proved somehow resistant to a second challenge with HIV (data not shown). In this regard, it is of interest to mention that human anti-HIV-1 antibodies were frequently detected in the sera of mice infected with HIV-1 and treated with CIFN. These observations suggest that CIFN can efficiently restrict HIV-1 replication in hu-PBL-SCID mice not only by a direct induction of an antiviral state in the human target cells, but also by stimulating anti-HIV human immune mechanisms. Although further studies are necessary to characterize the nature of the IFN-induced human "immune" mechanisms in HIV-1-infected hu-PBL-SCID mice, these results are consistent with an increasing number of reports indicating that type I IFN can act as a powerful T cell adjuvant for the generation of a protective immune response to tumor growth and virus infections (for reviews, see Belardelli and Gresser, 1996; Belardelli, 1995). In fact, while the assumption that IFN-α can be an immunosuppressive factor is based mostly on *in vitro* studies on the IFN-induced inhibition of T cell activation under certain experimental conditions (Zagury *et al.,* 1998), some *in vivo* studies have reported that this cytokine can enhance the *in vivo* proliferation of certain T cell subsets in mice (Tough *et al.,* 1996). Although the *in vivo* enhancement of T cell proliferation by type I IFN in mice was generally observed for CD8 T cells (especially $CD44^{hi}$) (Belardelli *et al.,* 1998; Tough *et al.,* 1996), an increase in the proliferation of CD4 T cells was also detected in animals injected with cells expressing IFN-α (Belardelli *et al.,* 1998). Moreover, studies in human cell systems indicate that IFN-α can enhance the differentiation of T cells toward a Th-1 type of immune response (Rogge *et al.,* 1997; Romagnani, 1992). In this regard, it is worth mentioning that recent studies on genetically modified human PBL expressing constitutive low levels of IFN-β show that this cytokine not only can suppress HIV replication, but also can enhance an anti-HIV T cell reactivity, which is associated with the promotion of a Th-1 type of immune response (Vieillard *et al.,* 1997). Recent results also indicate that type I IFN is an important stimulator of differentiation and activity of dendritic cells (Luft *et al.,* 1998; Paquette *et al.,* 1998). All these data, together with the results of the present study, indicate that type I IFN can act as an important stimulator of T cell functions, rather than a T cell suppressive factor, and suggest that therapeutic interventions including IFN-α should be considered for the prevention and therapy of HIV infection. Finally, our results emphasize the risk of extrapolating results obtained under *in vitro* conditions to clinical applications in patients and emphasize the importance of using animal models for the definition of selective strategies to prevent HIV-1 infection and the virus-induced disease.

MATERIALS AND METHODS

Animals. CB17 scid/scid female mice (Arlam, Nossan, Italy) were used at 4 weeks of age and were kept under specific pathogen-free conditions. SCID mice were housed in microisolator cages and all food, water, and bedding were autoclaved prior to use.

Reagents. Monoclonal anti-mouse granulocyte antibodies (RB6-8C5 hybridoma) were prepared as previously described (Santini *et al.,* 1998). Type I consensus IFN (Alton *et al.,* 1983) is a synthetic IFN produced from recombinant DNA, whose sequence is based on a consensus derived from the amino acid sequences of the most common types of human IFNα. CIFN had a specific activity of 1×10^9 units/mg protein and was kindly provided by Amgen (Thousand Oaks, CA). IFN was ti-

trated on HeLa cells as described elsewhere (Zhang *et al.,* 1996). AZT (Sigma–Aldrich, Milwaukee, WI) was dissolved in prewarmed (40°C) PBS at a concentration of 9 mg/ml and filter-sterilized. Mice were daily injected ip with 1 ml of a freshly prepared AZT solution [480 mg/kg/day (maximal tolerated dose)] or sc with 0.2 ml of CIFN (10^5 U/mouse).

Genetically modified U937 cells expressing the Tat-inducible IFN-A2 gene and control transduced cells. The origin and characteristics of HIV-1-LTR-IFNA2 U937 (clone ULTR5-14) cells have been described elsewhere (Su *et al.,* 1995). Genetically modified U937 cells transduced with the HIV-LTR control construct were used as controls. Previous studies have shown that HIV-1 replication was restricted *in vitro* in the U937 cells transduced with the Tat-transactivable IFN-A2 gene compared to control cells (Poli *et al.,* 1989). HIV-1-LTR-IFNA U937 cells produced low levels (80 U/ml) of IFN-A2; HIV-1 infection consistently stimulated IFN production (Su *et al.,* 1995).

Growth of U937 cell tumors in SCID mice and in vivo HIV-1 infection (U937-SCID mouse model). The animal studies were performed in Biosafety Level 3 facility. Mice were injected sc in the shoulder with 20×10^6 uninfected U937 cells (Lapenta *et al.,* 1997) resuspended in 0.2 ml saline. The two major diameters of each tumor nodule were measured by caliper and the mean tumor diameter of each tumor was calculated. To deplete animals of some residual reactivity, SCID mice were injected ip with 0.2 ml (100 μg/mouse) of an anti-mouse granulocyte mAb, 1 day before and 3 and 7 days after tumor cell injection (Santini *et al.,* 1998). The *in vivo* HIV infection of U937 SCID mice was performed using two different approaches: (i) simultaneous sc injection of 20×10^6 uninfected U937 cells with 10^6 $TCID_{50}$ of cell-free HIV-1 IIIB; (ii) peritumoral injection of established (15 days postimplantation) uninfected U937 cells tumors with 10^6 $TCID_{50}$ of cell-free HIV-1 IIIB.

Detection of viral infection in the U937-SCID model. At sacrifice, the HIV-infected chimeras were analyzed for the virus replication at the tumor site and p24 antigenemia. In particular, the U937 cell tumors were excised, carefully minced, and gently stirred for 30 min at 37°C in trypsin–EDTA solution (ICN Pharmaceuticals, Amsterdam, The Netherlands) to obtain cell suspensions. Cell suspensions underwent (a) HIV-1-DNA PCR as previously described (Lapenta *et al.,* 1997; Rizza *et al.,* 1996); (b) immunocytochemistry for the expression of HIV-p55/p18 (Lapenta *et al.,* 1997); or (c) cocultivation with C8166 cells for syncytia formation. Sera of infected animals were tested for HIV p24 antigen by an antigen capture enzyme-linked immunosorbant assay (Dupont, B-1130 Brussels, Belgium), for HIV-1 RNA copies by nucleid acid sequence-based amplification assay (NASBA HIV-1 RNA QT kit, Organon Teknika, Turnhout, Belgium), and also for syncytia formation in C8166 cell cultures.

Immunocytochemistry. Cells were obtained by disaggregation of tumors grown in infected SCID mice, attached to L-poly-L-lysine-covered glass chamber slides (Labtek, Naperville, IL) and stained by immunocytochemistry for HIV-1 p55/p18 matrix protein (IgG1, clone 11H9, from Medical Research Council AIDS Reagent Project, London, UK) using the alkaline phosphatase anti-alkaline phosphatase (Dako, Denmark) method.

The hu-PBL-SCID mouse model. Hu-PBLs were obtained from the peripheral blood of healthy donors. All donors were screened for HIV-1 and hepatitis prior to donation. The hu-PBLs were obtained by Ficoll-Paque density gradient centrifugation. Twenty million cells were resuspended in 0.5 ml RPMI 1640 medium and injected ip into the recipient mice. At different times after hu-PBL injection, SCID mice were sacrificed and cells were collected from the peritoneal cavity, spleen, and lymph nodes. At each time, a two-step peritoneal lavage was done. The first washing was performed with 1 ml of cold RPMI 1640 medium. The recovered volume was centrifuged and the supernatant was stored at −20°C, while the cells were pooled with those obtained with a second 4-ml washing.

ELISA for human immunoglobulins. An ELISA system was used to quantitate human IgG, IgM, and IgA in peritoneal lavage and sera of the chimeras using a goat anti-human F(ab)2 Ig antibody and peroxidase-coupled goat anti-human IgG, IgM, and IgA (Cappel-Cooper Biomedical, West Chester, PA). All ELISAs were performed in duplicate and laboratory standards were included on each plate. Peritoneal lavages and sera from nonreconstituted SCID mice were used as negative controls of all the ELISAs determinations. ELISA for detection of specific anti-HIV antibodies was performed as described in detail elsewhere (Ausiello *et al.,* 1994) using a specific peptide (i.e., ERYLKDQQLLGIWGCSGKLIC) corresponding to amino acids 591 to 611 of the gp41 HIV-1 protein. Synthetic peptides were immobilized on Maxi Sorp (NUNC) microtiter plates by an overnight incubation at 4°C with the peptide solution (100 μl/well; 2 mg/ml in water). Diluted mouse sera were added and incubated for 90 min at room temperature. Finally, binding was revealed by reading A_{450} values after a 15-min incubation at room temperature with *o*-phenylenediamine as a substrate. Values represent the mean adsorbance value of each individual serum tested in duplicate. The cut-off value was calculated as the mean adsorbance value of all the control sera. Sera showing A_{450} values higher than this threshold were considered positive for anti-HIV antibodies.

Flow cytometric analysis. Cells recovered from the peritoneum of the hu-PBL-SCID mice were resuspended in PBS and incubated with the appropriate fluorochrome-conjugated mAbs for 30 min. Cells were then washed with 2% PBS, 0.1% FCS, sodium azide and fixed with 2.5% paraformaldehyde. Two-color flow-cytometry was per-

formed on a FACSort cytometer (Becton Dickinson, San Jose, CA), and stained cells were analyzed using LYSIS II (Becton Dickinson) software. A total of 5000 events/sample were collected. Cells were analyzed according to forward- and side-scatter properties to gate the live cell population, allowing for the exclusion of erythrocytes, dead cells, and cell and tissue debris. The mAbs used were anti-human CD3, CD4, and CD8 (Becton Dickinson). None of the mAbs to human antigens showed cross-reaction to murine epitopes.

Evaluation of HIV-1 infection in the hu-PBL-SCID mouse model. For *in vivo* infection, 10^3 $TCID_{50}$ of HIV-1 SF162 were injected ip in hu-PBL-SCID mice 2 weeks after engraftment. The chimeras were sacrificed at 4–6 weeks after reconstitution and analyzed for HIV-1 infection by (i) DNA-PCR for viral specific sequences and for detection of the HLA-DQα, as described elsewhere (Rizza *et al.,* 1996) and (ii) p24-antigen detection in peritoneal lavage by ELISA (Dupont, B-1130). Sera of infected animals were tested for HIV-1 RNA copies by nucleid acid sequence-based amplification assay (NASBA HIV-1 RNA QT kit, Organon Teknika).

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